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Lethal Effect of Gamma Irradiation on *Euglena* Cells in Two Different Growth Phases

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Growth response and lethal effect of *Euglena* cells exposed to 1×10^5 r gamma-rays were investigated in two different growth phases, logarithmic and stationary. The logarithmic phase cells showed characteristic features different from the stationary phase cells in the following five points: first, the irradiated logarithmic phase cells were small in death rate than the irradiated stationary phase cells; second, the former cells showed more rapid growth recovery than the latter ones; third, the former cells were more few in sluggish cell number than the latter ones; fourth, the former cells were more small in degree of swelling than the latter ones; and fifth, the former cells were less in water content than the latter ones. The dry weight of all the cells used was hardly affected by irradiation and remained almost unchanged during the incubation period after irradiation.

INTRODUCTION

The irradiation effect on cell growth is one of the most essential problem in radiation biology. However, there are two points of view in the so-called cell growth. One means an increase in cell number, the other an increase in dry weight of a single cell. But, only a little is known at present about the ionizing radiation effect on cell growth from these viewpoints. It is well known that protozoa and bacteria are more resistant to ionizing radiation than most cells of multicellular organisms and that they do not show any division delay or any growth inhibition until they are irradiated with a considerably high dose of radiation (Baco and Alexander, 1955¹⁾; Kimball and Vogt-Köhne, 1962²⁾). Accordingly, as reported by Dognon and Piffault, (1931)³⁾ and Halberstaedter and Back, (1942)⁴⁾, these cells must require a very high dose for a lethal dose.

As to the irradiation effect on cell growth, it seems reasonable to consider the effect from the following two points. First, radiosensitivity of a given cell may vary with its age (Karnofsky *et al.*, 1950⁵⁾; Wilson and Karr, 1950⁶⁾). In other words, different stages in life cycle of a given cell may exhibit different irradiation effects on its growth (Tang and Gaw, 1937⁷⁾; Brown *et al.*, 1933⁸⁾). For instance, in animal cells, Karnofsky *et al.*, (1950) have reported that x-ray irradiated chick embryo manifests an increasing sensitivity to lethality during the third to the seventh day of incubation, and is most sensitive on the eighth to the tenth day, then after its response to irradiation is stabilized at a slightly more resistant level. In unicellular organism, Tang and Gaw, (1937)⁷⁾ have found that older culture

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of *Paramecium bursaria* is more susceptible to immediate lethal effects of ultra-violet light than younger one. Stapleton (1952)⁹⁾ has reported that x-ray irradiated *Eschericia coli* from fully grown culture in the stationary phase yields exponential survival curves. He has also reported that when the cells in the lag phase are exposed to x-rays, sigmoidal survival curves are obtained. Second, all the above mentioned protozoa and bacteria are heterotrophic organisms. With regard to autotrophic organisms, specially unicellular algae, the irradiation effect on growth has hardly been investigated as yet.

The purpose of the present study is to obtain some information on the irradiation effect on growth of unicellular algae. For this purpose, *Euglena* cells were chosen as the present material, because they increase in number by cell fission and do not show any complicated life cycle, such as conjugation or karyomixis.

MATERIAL AND METHODS

Cells, Media, and Conditions for Culture and Irradiation

Euglena gracilis var. *bacillaris*, Camb. Coll. No. 1224/7, in two different growth phases of culture, logarithmic and stationary was used throughout the present work. When about 4×10^6 stock cells were cultured for 5 day in 1500 ml of fresh culture medium under artificial light (about 2500 lux) at 30°C without shaking and aeration, these cells were taken as the "logarithmic phase cells," and the cells cultured for 20 days as the "stationary phase cells" in the present study. The culture medium used was a diluted one of Zumstein's medium.¹⁰⁾ That is, the medium contains 1.7 g pepton, 1.7 g glucose, 1.0 g citric acid, 0.2 g magnesium sulfate, and 0.5 g monopotassium phosphate in 1500 ml of distilled water (pH 3.4). This culture medium is used to give the most rapid growth of the cells among many kinds of nutrient solution proposed.^{11,12)} Consequently, though *Euglena* cells are autotrophic, this pepton nutrient solution was used in the present work.

The cells were harvested centrifugally from the cultured cell suspension and washed with fresh culture medium. The washed cells were suspended in 100 ml of fresh culture medium. This cell suspension was divided into equal halves, and each of which was enclosed into a polyethylen capsule. One capsule containing about 5×10^6 cells of either growth phase was exposed to 1×10^5 r gamma-rays, while the other capsule was kept as the control. The irradiation dose used was that used by Leedale and others.^{13,14)} The gamma-ray irradiation facility of the Institute for Chemical Research of Kyoto University was used. The dose rate used for the present work was 1.01×10^5 r/hr. Immediately after the irradiation, the irradiated and non-irradiated cell suspensions were transferred into each 1500 ml of fresh culture medium from the capsule and incubated for 14 days in the same manner as described above. From these incubated cell suspension, a certain volume was removed and used for investigations as needed.

Estimation of Cell Number

One ml of the cultured cell suspension homogenated by shaking was diluted

to one liter with distilled water, until one drop contained about 5 to 10 cells in most cases. Hundred drops of the diluted suspension were placed on a slide glass by means of a micropipette. The volume of a drop was $5\mu\text{l}$, and the number of cells found in the drop was determined under a microscope and then that of the original volume of culture medium, was calculated.

Dead Cell

Two methods of dead cell estimation were carried out in the present study ; hanging drop culture and methylen blue staining methods. In the former method, one drop of cell suspension containing 2 or 3 cells was incubated for 24 hours, because the lag phase of the cells requires about 7 to 8 hours in the same culture conditions as above. Thus, the cells which showed no swimming and no cell division at all were counted as "dead cells." In the latter method, the cells which were immediately stained with methylen blue after the addition of 1 % dye solution were taken as the "dead cells" in the present work.

Cell Size and Volume

As it was difficult to measure the size of an actively swimming cell without any treatment, the cells were fixed with 10 % aqueous formalin solution, and immediately after the fixation, the length and width of the fixed cells were measured with a micrometer. Moreover, as most fixed cells were nearly ellipsoidal, an individual volume of these cells was calculated assuming the cell shape as an ellipsoid.

Fresh and Dry Weight of Cell

A known number of the cells incubated was gathered by filtration on a small glass filter of type 12G3 and then washed thoroughly with distilled water. After the washing, the distilled water was eliminated with a suction pump. After that, the glass filter was weighed together with the washed cells. From the weight measured, the weight of the glass filter itself was reduced. Thus, the fresh weight of the cells was determined, and from this value the fresh weight per cell was calculated. Then the glass filter containing the fresh cells was desiccated at 110°C for 1 hr. After cooling, its weight was weighed again. The total dry weight of the cells was obtained by subtracting the weight of the glass filter, and the dry weight per cell was calculated as mentioned above.

RESULTS

1) Growth

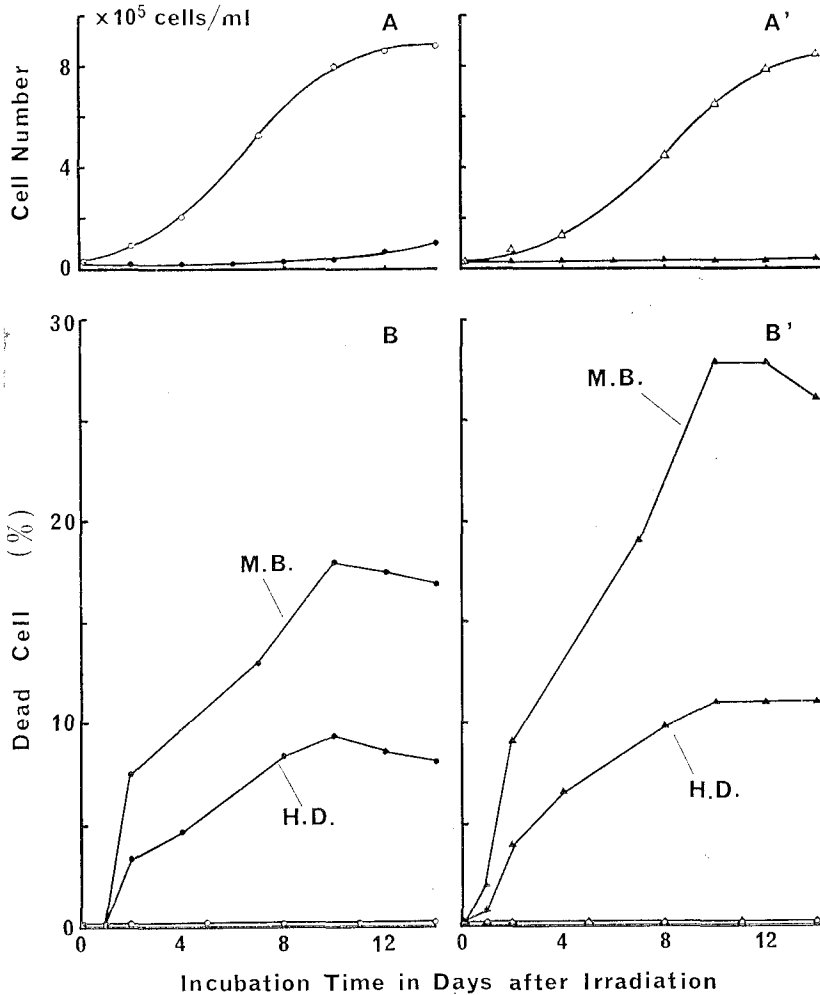
a) **Cell Number.** When about 5×10^6 non-irradiated logarithmic phase cells were incubated for 14 days in 1500 ml of fresh culture medium at 30°C , the cells rapidly increased in number and reached the stationary phase of culture on about the 12th days (Fig. 1, A ; $-\bigcirc-$). When the irradiated cells were incubated in the same manner as above, the cells remained unchanged in number for about 7 days and then began to gradually increase according to incubation time (Fig. 1, A ; $-\bullet-$).

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On the other hand, when about 5×10^6 non-irradiated stationary phase cells were cultured, the cells gradually increased in number for the first 4 days of culture and rapidly on near the 8th day and reached the stationary phase of culture on about the 14th day (Fig. 1, A'; $-\triangle-$). Contrary to this, the irradiated stationary phase cells were entirely inhibited in growth for about 13 days, but these cells showed a tendency to increase gradually in number in the culture for about 14 days or more (Fig. 1, A'; $-\blacktriangle-$).

b) **Dry Weight per Cell.** When the non-irradiated logarithmic phase cells were incubated in fresh culture medium, the dry weight per cell of these cells remained without a marked change during the incubation period. The average dry weight per cell of these cells was about 5.5×10^{-10} g. When the irradiated cells were incubated, these cells were hardly affected in dry weight per cell, and remained unchanged in dry weight during the incubation period (Fig. 1, E; $-\circ-$, $-\bullet-$).

When the non-irradiated and irradiated stationary phase cells were incubated,



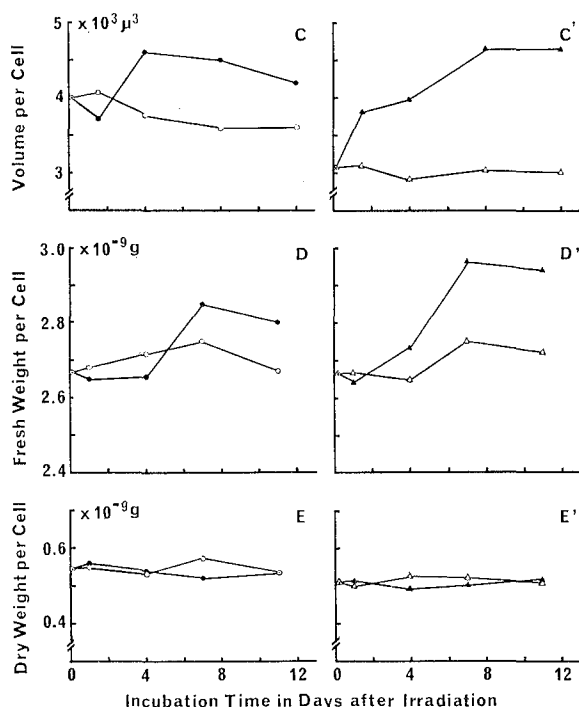


Fig. 1. Logarithmic phase cells (Figs. A to E) ; non-irradiated (—○—) and irradiated (—●—), Stationary phase cells (Figs. A' to E') ; non-irradiated (—△—) and irradiated (—▲—). A and A' ; increase in cell number. B and B' ; frequency of dead cells, H. D. ; Observation by hanging drop culture method, M. B. ; Observation by methylen blue staining method. C and C' ; volume per cell. D and D' ; fresh weight per cell. E and E' ; dry weight per cell.

respectively, in the same manner as above, the dry weight per cell of these cells also remained unchanged during the incubation period. The irradiation effect on dry weight was also not recognized. The average dry weight per cell of the non-irradiated stationary phase cells was about $5.1 \times 10^{-10} \text{g}$. But a significant difference of dry weight per cell was hardly recognized between the non-irradiated logarithmic and stationary phase cells. Consequently, a net increase of dry weight per cell, which is related to the individual growth of the cells was not recognized in the present work (Fig. 1, E' ; —△—, —▲—).

2) Dead Cells

No dead cell, of course, was observed in all the culture media of the non-irradiated cells of both growth phases in the present study. When the irradiated logarithmic phase cells were cultured, the dead cells induced by irradiation were hardly recognized for the first day after irradiation. They came to appear in some degree on the second day after irradiation, and then increased in number according to incubation time, and reached the maximum in percentage on about the 10th day of culture. The maximal percentage was about 9% in the hanging drop culture method, and about 18% in the methylen blue staining method (Fig. 1, B ; —●—).

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When the irradiated stationary phase cells were cultured as above, the tendency of dead cell appearance was similar to that of the irradiated logarithmic phase cells. The maximal percentage of dead cells was also recognized on about the 10th day of culture. It was about 11 % in the hanging drop culture method, and about 28 % in the methylen blue staining method (Fig. 1, B' ; -▲-).

The death rate of the irradiated cells of both growth phases decreased in the later period of culture. This decrease is a seeming one, because the irradiated cells begin to increase in number on about the 8th day after irradiation for the logarithmic phase cells and on about the 14th day for the stationary phase cells. Consequently, the ratio of the dead cells to the living cells decreases according to incubation time of culture.

On the other hand, when the non-irradiated cells of both growth phases were cultured in fresh medium, respectively, sluggish cells, inactively swimming ones, were observed in small number (1 to 2 %) for the logarithmic phase cells throughout the present incubation period of culture, but in large number (10 to 18 %) for the stationary phase ones.

When the irradiated cells of both growth phases were cultured in the same manner as above, the cells were sluggish in swimming by about 69 % on the first day of culture after irradiation in the irradiated logarithmic phase cells, and by about 89 % in the irradiated stationary phase ones. These percentages decreased according to incubation time. The difference of sluggish cell percentages of both irradiated growth phase cells was significant at 5 % level in the beginning of culture after irradiation.

3) Volume

As shown in Table 1, the difference in cell length was hardly recognized between both non-irradiated growth phase cells. But the width of the non-irradiated logarithmic phase cells was only a little larger than that of the non-irradiated stationary phase cells. Moreover, no difference of cell size was also recognized between the cells of both irradiated growth phases. But a little difference was recognized between the non-irradiated cells and the irradiated ones in both growth phases. That is, the irradiated cell length became a little shorter than the non-irradiated cell one. Further, the cell width of the former cells became somewhat wider than that of the latter ones. These phenomena suggest that an ellipsoidal

Table. 1. Size and volume of *Euglena* cell fixed with 10 % formalin.

	Logarithmic phase cells		Stationary phase cells	
	Non-irradiated	Irradiated	Non-irradiated	Irradiated
L×W (μ)	32.1×151 ±(5.5×1.8)	29.5×17.5 ±(4.3×1.6)	32.4×13.3 ±(4.4×1.4)	27.5×17.3 ±(3.1×1.6)
W/L	0.47	0.61	0.42	0.63
Volume (μ ³)	3833.3	4571.4	3092.6	4310.4

L ; cell length, W ; cell width. The cells measured were incubated for 7 days after irradiation. The cell volume was calculated assuming the cell shape as an ellipsoid. Each value of cell length or width was an average of about 500 cells.

form of the cells approaches to a spherical one. In fact, the cells of almost perfectly spherical form were frequently observed in the culture medium incubated for one day or more after the irradiation.

The volume of the non-irradiated logarithmic phase cells was generally larger than that of the non-irradiated stationary phase cells. When the non-irradiated cells of both growth phases were incubated in fresh culture medium respectively, the volume of these cells decreased according to incubation time in the logarithmic phase cells (Fig. 1, C ; \circ), but remained almost unchanged during the same incubation time, as above, in the stationary phase cells (Fig. 1, C' ; \triangle).

When the irradiated logarithmic phase cells were incubated in the same manner as above, these cells temporarily decreased in volume by about 9 % at about 30 hours after irradiation, and then began to increase in volume, and reached a maximal volume on the 4th day after irradiation. The maximal volume was larger by about 20 % than that of the non-irradiated cells. Thereafter, the above cell volume gradually decreased according to incubation time (Fig. 1, C ; \bullet). When the irradiated stationary phase cells were incubated as above, the cells increased in volume according to incubation time, and reached the almost same maximal volume as that of the irradiated logarithmic phase cells on the 8th day of culture. The maximal volume was larger by about 52 % than that of the non-irradiated cells. After that, the maximal volume remained unchanged during the present culture period (Fig. 1, C' ; \blacktriangle).

4) Water Content

When the non-irradiated logarithmic phase cells of about 2.68×10^{-9} g per cell in fresh weight were incubated in fresh culture medium, the fresh weight per cell of these cells increased according to incubation time and reached a maximum on the 7th day, then it decreased (Fig. 1, D ; \circ). When the irradiated cells of the same fresh weight as above were incubated, the fresh weight per cell of these cells decreased a little than that of the non-irradiated cells on the first day after irradiation, and then remained unchanged for about 3 days. But after that, it began to increase and reached a maximum on the 7th day, then decreased according to incubation time as above (Fig. 1, D ; \bullet).

On the other hand, when the non-irradiated stationary phase cells of about 2.66×10^{-9} g per cell in fresh weight were incubated in the same manner as above, the fresh weight of these cells remained almost unchanged at least in the first 4 days of culture. Then, it began to increase, and reached the maximum on the 7th day (Fig. 1, D' ; \triangle). When the irradiated cells were incubated, the fresh weight per cell decreased temporarily a little than that of the non-irradiated cells. But it rapidly increased thereafter, and reached the maximum on the 7th day (Fig. 1, D' ; \blacktriangle).

As mentioned above, the dry weight of the cells remained unchanged during the culture period. Accordingly, when the water content of the cells was calculated, an average percentage of water content was about 79.7 % of fresh weight in the non-irradiated logarithmic phase cells, about 80.1 % in the irradiated logarithmic phase cells, about 80.9 % in the non-irradiated stationary phase cells,

and about 81.7 % in the irradiated stationary phase cells. Although the water content did not change markedly during the culture period in the non-irradiated cells of both growth phases, in the irradiated cells it showed a tendency to increase slightly in the later period of culture. From this result, it was assumed that the cell permeability to water would change after the irradiation.

DISCUSSION AND CONCLUSION

When *Euglena* cells of two different growth phases, logarithmic and stationary, of culture were irradiated with 1×10^5 r gamma rays and cultured, thereafter some of the irradiated cells are damaged to death, and the survivals are inhibited in growth. In spite of the high irradiation dose used in the present work, the dead cells induced were unexpectedly small in quantity, that is, below about 30 % of the total irradiated cells in both growth phase cells. This result may show that *Euglena* cells are fairly radioresistant. This conclusion may be reasonable, because it is known that the ciliated protozoa are more resistant to the division delaying or blocking action of ionizing radiation than most cells of multicellular organisms.^{1,2,15)}

Further, it is well known that a rapidly growing cell of multicellular organisms is more radiosensitive than a slowly or non-growing cell.^{15,19)} But only a little is known as to the radiosensitivity of unicellular algae. For instance, Davies¹⁷⁾ has reported that the rate of recovery of gamma-irradiated *Chlamydomonas* varies slightly at different times in the cycle and is rapid immediately after irradiation than at later times. Stapleton⁹⁾ has made an observation that the form of the survival curve obtained after x-ray irradiation of *E. coli* cells depends on the stage of the growth cycle of the culture. That is, the irradiated lag phase cells yield sigmoidal curves, while the irradiated stationary phase cells exponential ones. These observations, as well as the results obtained in the present work, may be taken to contradict the above mentioned view. In the present work, the logarithmic phase cells of *Euglena* are understood to be more resistant to gamma-irradiation than the stationary phase cells, because the dead cell number produced by irradiation was less in the irradiated logarithmic phase cells than in the irradiated stationary phase ones. This death rate may be taken as a direct indicator of radiosensitivity of the cells. Further, there are some evidences to show that the logarithmic phase cells are more resistant to radiation. First, the irradiated logarithmic phase cells began to recover from the growth inhibition more rapidly than the irradiated stationary phase cells. Second, the former cells were less in sluggish cell number than the latter cells.

The individual growth of irradiated cells, which was pointed out in *E. coli* by Lea *et al.*¹⁸⁾ was not observed in the present work. They have reported that *E. coli* irradiated with a small dose of gamma rays increases in cell size during the division delaying period. This may be taken as an evidence to show the individual growth of irradiated cell. In the present work the irradiated *Euglena* cells surely increase in fresh weight and in cell volume during the culture period after irradiation. But the dry weight of these cells remained unchanged from that of the non-irradiated cells during this period. Consequently, any increase in

dry weight of single cell, presumably by protein synthesis, of irradiated individual cell could not be recognized. The increase of cell volume and fresh weight mentioned above seems to depend on the change of cell permeability to water after irradiation. Srb and Hluchofsky¹⁹⁾ have showed by means of plasmolysis that the epidermal cell permeability of *Allium cepa* exposed to x-rays is changed. Kimball and Vogt-Köhne²⁾ have observed that the rate of food vacuole formation increases during the interdivision interval in x-ray irradiated *Paramecium aurelia*.

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